Effect of Cry3Bb1-Expressing Transgenic Corn on Plant-to-Plant Movement by Western Corn Rootworm Larvae (Coleoptera: Chrysomelidae)

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ABSTRACT Dispersal of larvae of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, in specific combinations of transgenic corn expressing the Cry3Bb1 protein and nontransgenic, isoline corn was evaluated in a 2-yr field study. In total, 1,500 viable western corn rootworm eggs were infested in each subplot. Each year, plant damage and larval recovery were evaluated among four pedigree combinations (straight transgenic; straight nontransgenic corn; nontransgenic corn with a transgenic central, infested plant; and transgenic corn with a nontransgenic central, infested plant) on six sample dates between egg hatch and pupation. For each subplot, the infested plant, three successive plants down the row (P1, P2, and P3), the closest plant in the adjacent row of the plot, and a control plant were sampled. The number of western corn rootworm larvae recovered from transgenic rootworm-resistant plants adjacent to infested nontransgenic plants was low and not statistically significant in either 2001 or 2002. In 2001, significantly fewer larvae were recovered from transgenic rootworm-resistant plants than from nontransgenic plants when both were adjacent to infested, nontransgenic plants. In 2002, significantly more neonate western corn rootworm larvae were recovered from nontransgenic plants adjacent to infested, transgenic rootworm-resistant plants than nontransgenic plants adjacent to infested, nontransgenic plants on the second sample date. Together, these data imply that both neonate and later instar western corn rootworm larvae prefer nontransgenic roots to transgenic rootworm-resistant roots when a choice is possible. However, when damage to the infested, nontransgenic plant was high, western corn rootworm larvae apparently moved to neighboring transgenic rootworm-resistant plants and caused statistically significant, although only marginally economic, damage on the last sample date in 2001. Implications of these data toward resistance management plan are discussed.

KEY WORDS larval movement, resistance management, transgenic, *Bacillus thuringiensis*, *Diabrotica virgifera virgifera*

TRANSGENIC CORN, *Zea mays* L., that expresses endotoxins from the soil bacterium *Bacillus thuringiensis* Berliner (Bt), has been developed by several seed companies to control damage from larvae of the western, *Diabrotica virgifera virgifera* LeConte, and northern, *Diabrotica barberi* Smith & Lawrence, corn rootworm (English et al. 2000, Moellenbeck et al. 2001, Ellis et al. 2002, EPA Scientific Advisory Panel 2002, Baum et al. 2004, Vaughn et al. 2005). Transgenic corn expressing the Cry3Bb1 endotoxin was grown on >800,000 ha in 2004. Other transgenic events targeting the corn rootworm complex are in the registration process.

As part of the registration process for Bt crops, registrants must submit an insect resistance management (IRM) plan to the Environmental Protection Agency (EPA). Development of an appropriate IRM plan for transgenic corn hybrids that control corn rootworms must include, among other things, an understanding of important biological parameters such as larval movement and dispersal. Hibbard et al. (2003) demonstrated that larvae could move at least three plants down a row and across a 0.46-m row after initial establishment on a plant. Hibbard et al. (2004) demonstrated that larvae tended to say on the infested plant when little damage had occurred and moved primarily after significant damage had occurred to the infested plant. Although it was clear from this research that larvae had the potential to move under certain conditions, it was not clear how other factors might influence larval dispersal, such as soil type, other plant species, or the presence of transgenic corn plants.

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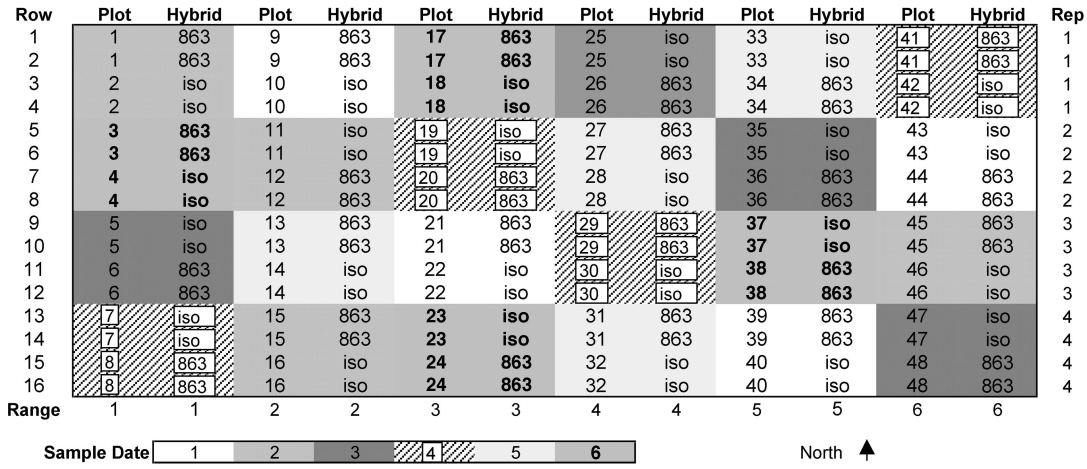

Fig. 1. Field layout for 2001. Each range was 9.15 m and was separated by a 1.22-m unplanted alley. Within each plot, four subplots were created in the north row. Two of the subplots had the infested plant being a corn pedigree different than the remainder of the plot. One of each subplot type was used for larval recovery and one for evaluating plant damage.

Movement of larvae from susceptible to transgenic plants expressing a high dose of toxin, and vice versa, has been hypothesized to adversely affect IRM in several ways (Mallet and Porter 1992, Davis and Onstad 2000). In Cry3Bb1, where the transgenic plant does not offer a high dose (EPA Scientific Advisory Panel 2002), larval movement may have several effects on the rate of resistance development, depending upon the genetic architecture of the resistant phenotype. Initial development on a susceptible plant (a grassy weed or corn plant) followed by subsequent migration to a nearby transgenic plant could accelerate the rate of adaptation if heterozygotes with the resistance gene survived exposure to the endotoxin at higher rates. Likewise, if larvae briefly fed on a transgenic root and then migrated to a nearby susceptible root, this, too, could accelerate the rate of resistance development if heterozygotes with the resistance gene were preferentially selected. However, later rootworm instars are much more tolerant to Cry3Bb1 endotoxins (EPA Scientific Advisory Panel 2002), so partial development on susceptible plants followed by movement to Cry3Bb1-expressing corn may simply result in the production of additional susceptible beetles from within the transgenic field. The goal of the current experiment was to evaluate whether transgenic rootworm-resistant corn expressing the Cry3Bb1 protein affects postestablishment movement by western corn rootworm larvae.

Materials and Methods

2001. The study was conducted at the University of Missouri Bradford Research and Extension Center, 9 km east of Columbia, MO, on a Mexico silt loam soil type. Soil composition at the site was determined to be 2% sand, 70% silt, and 28% clay (University of Missouri Soils Testing Lab). The field selected for use had been planted with soybean, *Glycine max* (L.) Merr., in the previous year, and unlike parts of the eastern Corn Belt, egg laying by western corn rootworm females outside of corn has not been detected in Missouri. Because of these two factors, we assumed that feral western corn rootworms would not be found in our plots, but we verified this with uninfested controls. The experimental design was a randomized complete block split-split-plot in space in which the factors were arranged as a 6 by 4 by 6 (sample date × pedigree combination × plant category) factorial as outlined in Steel et al. (1997). The main plot effect was sample date, the subplot effect was pedigree combination, and the sub-subplot was plant category (Figs. 1 and 2).

Plots were hand planted on 30 April by using a 20-cm plant spacing and 0.76-m rows. There were four replications representing a four row block across the entire field (Fig. 1). In each replication, there were six 9.15-m ranges separated by a 1.22-m unplanted alley. Within each replication and range, there were two main plots (two rows each) in which both rows were either transgenic rootworm-resistant plants expressing the Cry3Bb1 endotoxin or its isoline (nontransgenic plants of the genetic background from which the transgenic originated). Before planting, in two locations within each of the two-row plots, a position was marked with an orange stake. In this location, a seed of the opposite type (nontransgenic in a transgenic rootworm-resistant plot and vice versa) was planted instead of the seed for the remainder of the two-row plot. At plant emergence, four subplots were created in each plot. Two of the subplots were at the location of the orange stakes. Two additional subplots were created in other locations within the main plot where good germination of at least four plants in a row had occurred. The central plant for the two new subplots was marked with a white stake. In total, four pedigree combinations were evaluated: 1) straight nontransgenic; 2) straight transgenic; 3) nontransgenic with a transgenic central, infested plant; and 4) transgenic

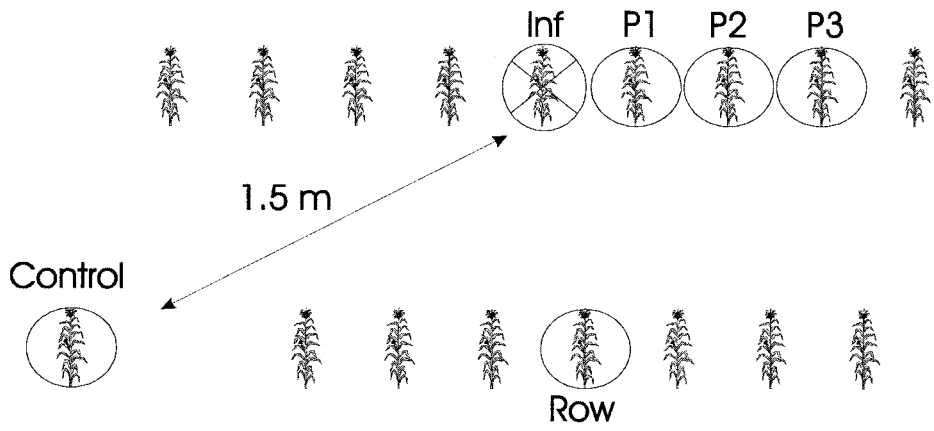

Fig. 2. Sampling plan within a subplot. The four pedigree combinations involved 1) all transgenic rootworm-resistant, 2) all nontransgenic, 3) the infested plant was transgenic rootworm-resistant and all others were nontransgenic, and 4) the infested plant was nontransgenic and all others were transgenic rootworm-resistant.

with a nontransgenic central, infested plant. Straight nontransgenic or transgenic pedigrees were those with white stakes in a nontransgenic or transgenic two-row plot, respectively. Nontransgenic with a transgenic, central plant and transgenic with a nontransgenic, central plant were the orange-staked plots in nontransgenic and transgenic two-row plots, respectively. Because transgenic seed availability was restricted in 2001 and 2002, only two main plots were actually planted, so the location of pedigree combinations 1–3 and pedigree combinations 2–4 were not random, and direct comparisons are not possible. These comparisons were made with a separate analysis (see Statistical Analysis). Subplots for the substituted seed were created at planting rather than at the V1–2 stage of plant development. In the few cases where a kernel did not germinate, plants of the correct pedigree were transplanted and watered at infestation time to maintain a uniform plant spacing of four plants in each subplot.

In total, 1,500 viable western corn rootworm eggs were infested on 15 May in each subplot, half on either side of each staked plant (between rows). Two petri dishes (100 by 15 mm, Fisher, Pittsburgh, PA) containing extra eggs and soil were wrapped in Parafilm and buried at infestation level away from any infested plots and were periodically dug up and examined to determine egg hatch initiation and duration. Within each main plot, one subplot of each colored stake was used for recovering larvae, and one subplot of each colored stake was used to evaluate plant damage. For each subplot, the infested plant, three successive plants down the row (P1, P2, and P3); the closest plant in the adjacent row of the plot; and a control plant at least 1.5 m from any infested plant, but also within the plot (six plants total), were sampled (Fig. 2). Enough plots were set up for four replications of root ratings and four replications of larval recovery at each of six sampling times. The range chosen for each sample date within a replication was randomly chosen from one of the six ranges available (Fig. 1). Sampling for both larval recovery and plant damage was destructive, so sampling the same plants over time was not possible with the techniques used.

Sampling began on 18 June, when larval hatch was first detected. Other sampling dates were 22 and 26 June and 2, 6, and 12 July. Each plant sampled was initially labeled with the plot location, subplot type, plant category, and a random code, but information on the plant category and subplot type was removed before evaluating for root damage or searching for larvae to eliminate any bias that might be possible if the pedigree and plant were known. Plant phenology also was recorded (Ritchie et al. 1992). Half of the subplots sampled were washed and rated for damage (Oleson et al. 2005). Root balls for the remaining half were placed in onion bags and hung over water pans (33 cm in diameter by ≈7.5 cm in depth) in a greenhouse bay (Hibbard et al. 2004). Western corn rootworm larvae falling from the onion bags into the water pans below were collected once or more (usually twice) on a daily basis and were stored in 95% ethanol until they could be processed. Roots were allowed to hang for larval recovery for a minimum of 7 d. Greenhouse temperatures were typically 38–50°C or more during the heat of the day. Most larvae were recovered within the first 4 d with this technique (usually peaking on days 2–4). Occasionally, when roots were sampled just after a rain and cool, cloudy days followed, larvae were still recovered on days 6 and 7. If this occurred, roots were allowed to hang for a longer period. During processing, each larva was first identified to species by using the key of Mendoza and Peters (1964) by close examination for the presence of urogomphi, (small protrusions on the posterior margin of the anal plate), which are present on southern corn rootworm larvae *D. undecimpunctata howardi* Barber, but not on western corn rootworm larvae (Mendoza and Peters 1964, Krysan 1986). The number of western corn rootworm larvae from each sample was counted, head capsule measurements were taken, and wet

Table 1. 2001 ANOVA tables for the no. of larvae recovered, plant damage, total wt of larvae recovered, and average weight of larvae recovered

Analysis	Effect	df[a]	*F* value	*P* > *F*
No. larvae	Replications	3, 15	1.62	0.2277
	Dates	5, 15	4.86	0.0077
	Plants	5, 360	31.58	<0.0001
	Dates × plants	25, 360	2.69	<0.0001
	Pedigree combination	3, 54	2.22	0.0966
	Dates × p.c.	15, 54	0.80	0.6748
	Plants × p.c.	15, 360	2.17	0.0071
	Dates × plants × p.c.	75, 360	0.69	0.9761
Larvae subtracted	Replication	3, 15	0.24	0.8664
	Dates	5, 15	0.30	0.9069
	Plants	5, 179	1.62	0.1570
	Dates × plants	25, 179	0.45	0.9895
	Pedigree combination	1, 18	4.48	0.0485
	Dates × p.c.	5, 18	1.43	0.2614
	Plants × p.c.	5, 179	3.62	0.0038
	Dates × plants × p.c.	25, 179	0.63	0.9142
Damage	Replications	3, 15	1.23	0.3324
	Dates	5, 15	14.13	<0.0001
	Plants	5, 360	39.09	<0.0001
	Dates × plants	25, 360	9.37	<0.0001
	Pedigree combination	3, 54	4.13	0.0104
	Dates × p.c.	15, 54	2.98	0.0017
	Plants × p.c.	15, 360	3.83	<0.0001
	Dates × plants × p.c.	75, 360	2.32	<0.0001
Damage subtracted	Replications	3, 15	0.21	0.8899
	Dates	5, 15	0.77	0.5882
	Plants	5, 179	0.93	0.4623
	Dates × plants	25, 179	1.27	0.1903
	Pedigree combination	1, 18	11.49	0.0033
	Dates × p.c.	5, 18	8.51	0.0003
	Plants × p.c.	5, 179	12.36	<0.0001
	Dates × plants × p.c.	25, 179	6.79	<0.0001
Avg wt	Replications	3, 12	0.32	0.8133
	Dates	5, 12	10.04	0.0006
	Plants	5, 3	1.57	0.3775
	Dates × plants	10, 3	0.66	0.7313
	Pedigree combination	3, 12	1.99	0.1690
	Dates × p.c.	14, 12	1.14	0.4159
	Plants × p.c.	6, 3	0.85	0.6043
	Dates × plants × p.c.	5, 3	1.45	0.4036

For "subtracted" ANOVAs, the number of larvae recovered (or damage) from each plant of the straight nontransgenic subplot were subtracted from the number of larvae recovered (or damage) from each plant of the nontransgenic with a transgenic infested plant subplot (infested minus infested, P1 minus P1, etc.). The same was done for the number of larvae recovered from the straight transgenic and transgenic with an infested nontransgenic rootworm-resistant plant. p.c., pedigree combination.

[a] Degree of freedom for the numerator, denominator.

weight was measured. Southern corn rootworm larvae were counted and discarded.

2002 Modifications. The experiment was repeated with several adjustments. First, we added an additional replication (five total). Second, the two plants that were the opposite plant type (nontransgenic plants in a transgenic subplot and vice versa) were transplanted at the V1–2 stage of plant development rather than substituting seed at planting. Transplanted plants were watered immediately and timely precipitation maintained their vigor. In addition, gene checks provided by Monsanto Company were run on all infested transgenic rootworm-resistant plants, all transgenic rootworm-resistant "P1" plants adjacent to an infested nontransgenic plant, and all infested nontransgenic plants in transgenic rootworm-resistant plots. The experiment was planted 19 April, infested at the V3 stage of plant development on 15 May after a cool late April and early May, and sampled on 12, 17, 20, 24, and 28 June, and 3 July. As in 2001, each larva was morphologically identified to species (Mendoza and Peters 1964). Because morphological data may not be effective in distinguishing between neonate western and neonate southern corn rootworm (Krysan 1986), and because of the large number of larvae morphologically identified as southern corn rootworms in 2002, we verified the effectiveness of our morphological separation by using polymerase chain reaction (PCR)-restriction fragment length polymorphism of a portion of the mitochondrial cytochrome oxidase subunit I (COI). Total DNA from 60 randomly selected larvae from the straight nontransgenic pedigree, 60 randomly selected larvae from the straight transgenic rootworm-resistant corn pedigree, 11 known western corn rootworm larvae (five) and adults (six), and eight known southern corn rootworm larvae (five) and adults (three) were extracted using a DNeasy tissue kit (QIAGEN, Valencia, CA)

Table 2. 2002 ANOVA tables for the no. of larvae recovered, plant damage, total weight of larvae recovered, and average weight of larvae recovered

Analysis	Effect	df[a]	*F* value	$P > F$
No. larvae	Replications	4, 20	2.29	0.0956
	Dates	5, 20	9.06	0.0001
	Plants	5, 480	45.01	<0.0001
	Dates × plants	25, 480	3.86	<0.0001
	Pedigree combination	3, 72	17.08	<0.0001
	Dates × p.c.	15, 72	2.45	0.0059
	Plants × p.c.	15, 480	11.25	<0.0001
	Dates × plants × p.c.	75, 480	1.50	0.0069
Larvae subtracted	Replications	4, 20	0.74	0.5737
	Dates	5, 20	0.20	0.9570
	Plants	5, 236	0.34	0.8886
	Dates × plants	25, 236	0.76	0.7911
	Pedigree combination	1, 24	22.12	<0.0001
	Dates × p.c.	5, 24	4.28	0.0063
	Plants × p.c.	5, 236	30.71	<0.0001
	Dates × plants × p.c.	25, 236	2.89	<0.0001
Damage	Replications	4, 20	0.16	0.9540
	Dates	5, 20	18.05	<0.0001
	Plants	5, 480	34.69	<0.0001
	Dates × plants	25, 480	5.07	<0.0001
	Pedigree combination	3, 72	35.04	<0.0001
	Dates × p.c.	15, 72	4.88	<0.0001
	Plants × p.c.	15, 480	16.23	<0.0001
	Dates × plants × p.c.	75, 480	2.46	<0.0001
Damage subtracted	Replications	4, 20	1.53	0.2315
	Dates	5, 20	1.16	0.3614
	Plants	5, 240	0.51	0.7701
	Dates × plants	25, 240	1.02	0.4369
	Pedigree combination	1, 24	69.44	<0.0001
	Dates × p.c.	5, 24	8.24	0.0001
	Plants × p.c.	5, 240	51.33	<0.0001
	Dates × plants × p.c.	25, 240	6.39	<0.0001
Avg wt	Replications	4, 19	0.81	0.5361
	Dates	5, 19	7.72	0.0004
	Plants	5, 101	2.33	0.0474
	Dates × plants	25, 101	1.54	0.0700
	Pedigree combination	3, 55	0.63	0.5983
	Dates × p.c.	15, 55	2.28	0.0138
	Plants × p.c.	15, 101	1.03	0.4346
	Dates × plants × p.c.	59, 101	1.07	0.3823

For "subtracted" ANOVAs, the number of larvae recovered (or damage) from each plant of the straight nontransgenic subplot were subtracted from the number of larvae recovered (or damage) from each plant of the nontransgenic with a transgenic infested plant subplot (infested minus infested, P1 minus P1, etc.). The same was done for the no. of larvae recovered from the straight transgenic and transgenic with an infested nontransgenic rootworm-resistant plant. p.c., pedigree combination.

[a] Degree of freedom for the numerator, denominator.

following the manufacturer's protocol for isolation of genomic DNA of insects. Following the methods of Clark et al. (2001a), a 625-bp portion of COI was amplified using the universal primers from the COI (C1-J-2441 5′-CCAACAGGAATTAAAATTTTTAGATGATTAGC-3′) and the tRNA leucine genes (TL2-N-3014 5′-TCCAATGCACTAATCTGCCATATTA-3′) (Simon et al. 1994) by using a GeneAmp PCR system 2700 (PerkinElmer, Branchburg, NJ). PCR amplification products (2 μl) were loaded onto 1.0% agarose TBE (0.089 M Tris, 0.089 M boric acid, and 0.5 M EDTA, pH 8.0) gels. After electrophoresis (75 V for 45 min), PCR products were visualized over a UV transilluminator and scanned into the Kodak 1D Image analysis software program (Eastman Kodak, Rochester, NY). PCR amplicons were then cut using the 4-bp recognition restriction endonuclease *Alu*I following the manufacturer's protocol (New England BioLabs, Beverly, MA) and methods described by Clark et al. (2001b). Digested PCR amplicons were fractionated (40 V for 4 h) on 2% agarose TBE gels and visualized over a UV transilluminator and scanned. Species determinations were made by comparing the PCR-restriction fragment-length polymorphism fragment profiles of known species identity to unknown samples.

Statistical Analysis. PROC MIXED of the statistical package SAS (SAS Institute 1990) was used for data analysis. A separate analysis was done each year for larval recovery, plant damage, and average larval weight. Data were analyzed as a randomized complete block split-split-plot in space outlined in Steel et al. (1997). Because the treatments were arranged as a 6 by 4 by 6 (sample date × pedigree combination × plant category) factorial, the linear statistical model contained the main plot effect of sample date, the subplot effects of pedigree combination and sampling date × pedigree combination, and the sub-subplot

Table 3. Number of western corn rootworm larvae ± SE recovered in 2001 from varying configurations of transgenic and nontransgenic plants when 1,500 viable eggs were placed on the infested (Inf) plant

Date	Plant	Transgenic	Trans w/non Inf	Nontrans	Nontrans w/trans Inf	Trans sub	Nontrans sub
18 June	Inf	0.0 ± 0.0bCm	0.8 ± 0.8bCm	4.8 ± 2.3aABm	0.3 ± 0.3bBm	0.8 ± 0.8aAm	−4.5 ± 2.1bBn*
22 June	Inf	14.5 ± 11.1abAm	20.3 ± 15.0aAm	9.3 ± 5.1abAm	5.3 ± 2.3bAm	5.8 ± 22.1aAm	−4.0 ± 7.1aCm*
26 June	Inf	7.5 ± 7.5aBm	5.1 ± 3.9aBm	6.8 ± 6.1aABm	0.0 ± 0.0bBm	−2.4 ± 8.8aAm	−6.8 ± 6.1bBCn*
2 July	Inf	2.5 ± 1.5aBm	2.0 ± 1.7aBCm	2.3 ± 1.7aBCm	0.5 ± 0.3aABm	−0.5 ± 2.9aAm	−1.8 ± 1.8aBCm
6 July	Inf	0.3 ± 0.3bBCm	1.3 ± 0.6bBCm	3.8 ± 1.3aABm	0.0 ± 0.0bBm	1.0 ± 0.8aAm	−3.8 ± 1.3bBn*
12 July	Inf	1.5 ± 1.5aBm	0.8 ± 0.8aCm	0.5 ± 0.3aCm	0.3 ± 0.3aBm	−0.8 ± 0.8aAm	−0.3 ± 0.3aACm
18 June	P1	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.0 ± 0.0aBn	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.0 ± 0.0aABm
22 June	P1	1.0 ± 0.7aAn	0.3 ± 0.3aAn	1.3 ± 0.8aABno	1.5 ± 1.2aAn	−0.8 ± 0.9aAn	0.3 ± 0.9aABm
26 June	P1	0.0 ± 0.0bAn	0.0 ± 0.0bAn	3.3 ± 2.4aAm	0.3 ± 0.3bAm	0.0 ± 0.0aAm	−3.0 ± 2.5aBmn*
2 July	P1	1.0 ± 0.6aAmn	0.0 ± 0.0aAn	0.0 ± 0.0aBn	0.5 ± 0.3aAm	−1.0 ± 0.6aAm	0.5 ± 0.3aAm
6 July	P1	0.0 ± 0.0aAm	0.3 ± 0.3aAmn	1.5 ± 1.5aABn	0.3 ± 0.3aAm	0.3 ± 0.3aAm	−1.3 ± 1.3aABm
12 July	P1	1.3 ± 0.8aAm	0.3 ± 0.3aAm	0.3 ± 0.3aBm	0.0 ± 0.0aAm	−1.0 ± 0.6aAm	−0.3 ± 0.3aABm
18 July	P2	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.0 ± 0.0aBn	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.0 ± 0.0aAm
22 June	P2	0.0 ± 0.0bAn	0.0 ± 0.0bAn	4.5 ± 4.5aAn	0.3 ± 0.3abAn	0.0 ± 0.0aAmn	−4.3 ± 4.6aAm
26 June	P2	0.0 ± 0.0aAn	0.5 ± 0.5aAn	1.3 ± 0.8aABmn	0.0 ± 0.0aAm	0.5 ± 0.5aAm	−1.3 ± 0.8aAmn
2 July	P2	0.0 ± 0.0aAn	0.3 ± 0.3aAmn	0.0 ± 0.0aBn	0.5 ± 0.5aAm	0.3 ± 0.3aAm	0.5 ± 0.5aAm
6 July	P2	0.0 ± 0.0aAm	0.0 ± 0.0aAn	0.3 ± 0.3aBn	0.0 ± 0.0aAm	0.0 ± 0.0aAm	−0.3 ± 0.3aAm
12 July	P2	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.0 ± 0.0aBm	1.0 ± 1.0aAm	0.0 ± 0.0aAm	1.0 ± 1.0aAm
18 July	P3	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.0 ± 0.0aAn	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.0 ± 0.0aAm
22 June	P3	0.0 ± 0.0aAn	0.0 ± 0.0aAn	0.0 ± 0.0aAo	0.3 ± 0.3aAn	0.0 ± 0.0aAmn	0.3 ± 0.3aAm
26 June	P3	0.0 ± 0.0aAn	0.0 ± 0.0aAn	0.0 ± 0.0aAn	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.0 ± 0.0aAm
2 July	P3	0.0 ± 0.0aAn	0.0 ± 0.0aAn	0.0 ± 0.0aAn	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.0 ± 0.0aAm
6 July	P3	0.0 ± 0.0aAm	0.0 ± 0.0aAn	0.3 ± 0.3aAn	0.0 ± 0.0aAm	0.0 ± 0.0aAm	−0.3 ± 0.3aAm
12 July	P3	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.0 ± 0.0aAm	1.3 ± 1.3aAm	0.0 ± 0.0aAm	1.3 ± 1.3aAm
18 June	Row	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.0 ± 0.0aAn	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.0 ± 0.0aAm
22 June	Row	0.3 ± 0.3aAn	0.0 ± 0.0aAn	0.0 ± 0.0aAo	0.0 ± 0.0aAn	−0.3 ± 0.3aAmn	0.0 ± 0.0aAm
26 June	Row	0.0 ± 0.0aAn	1.3 ± 1.3aAmn	0.0 ± 0.0aAn	0.0 ± 0.0aAm	1.3 ± 1.3aAm	0.0 ± 0.0aAm
2 July	Row	0.0 ± 0.0aAn	0.3 ± 0.3aAmn	0.0 ± 0.0aAn	0.3 ± 0.3aAm	0.3 ± 0.3aAm	0.3 ± 0.3aAm
6 July	Row	0.0 ± 0.0aAm	0.0 ± 0.0aAn	0.0 ± 0.0aAn	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.0 ± 0.0aAm
12 July	Row	0.0 ± 0.0aAm	0.5 ± 0.3aAm	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.5 ± 0.3aAm	0.0 ± 0.0aAm
18 June	Cnt	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.0 ± 0.0aAn	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.0 ± 0.0aAm
22 June	Cnt	0.0 ± 0.0aAn	0.0 ± 0.0aAn	0.0 ± 0.0aAo	0.0 ± 0.0aAn	0.0 ± 0.0aAmn	0.0 ± 0.0aAm
26 June	Cnt	0.0 ± 0.0aAn	0.0 ± 0.0aAn	0.0 ± 0.0aAn	0.3 ± 0.3aAm	0.0 ± 0.0aAm	0.3 ± 0.3aAm
2 July	Cnt	0.0 ± 0.0aAn	0.5 ± 0.3aAmn	0.0 ± 0.0aAn	0.0 ± 0.0aAm	0.5 ± 0.3aAm	0.0 ± 0.0aAm
6 July	Cnt	0.0 ± 0.0aAm	0.0 ± 0.0aAn	0.0 ± 0.0aAn	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.0 ± 0.0aAm
12 July	Cnt	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.0 ± 0.0aAm

Although untransformed data are shown, statistics were performed using log $(x + 1)$ data. Different uppercase letters indicate a significant difference within a column and plant. Different lowercase letters starting with "m" indicate a significant difference between plants, but within a column and date. Different lowercase letters starting with an "a" indicate a significant difference within a row (between treatments, but within a date and plant), either 1) among the third to sixth columns or 2) between the seventh and eighth column. However, because of the experimental design, specific comparisons of the third to fourth columns or the fifth to sixth columns are not appropriate. Significant differences for these comparisons are indicated by an * in the seventh or eighth columns, respectively (863 sub = column 4 minus column 3; Iso sub = column 6 minus column 5).

effect of plant category and all possible interactions with the main and sub-plot effects. Replications within dates served as the denominator of *F* for testing the effects of dates. Replications within pedigree combinations and sample dates served as the denominator of *F* for testing pedigree combination and the interaction of pedigree combination × sample date. All other effects used the residual error for the denominator. Beyond the standard analysis of variance (ANOVA), we preplanned to compare pedigree combinations within plant category and sampling dates, plant categories within pedigree combination and sampling dates, and sampling dates within pedigree combinations and plant category. This was done with the LSMEAN output from PROC MIXED (least significant difference [LSD] technique). Although untransformed data are shown in the tables, all data were transformed by log $(x + 1)$ for analyses to meet the assumptions of equal variance. Because average weight is total weight divided by the number of larvae, it only has meaning when larvae are recovered. Too many missing values were present in 2001, so average larval weight data are not reported for that year.

Because the straight transgenic rootworm-resistant corn treatment and the transgenic rootworm-resistant with a nontransgenic infested plant treatments were physically in the same main plot due to seed availability (as were straight nontransgenic and nontransgenic with an infested transgenic rootworm-resistant plant), direct comparisons of these pedigree combinations were not possible in the above-mentioned analysis. For these comparisons, the number of larvae recovered from each plant of the straight nontransgenic subplot was subtracted from the number of larvae recovered from each plant of the nontransgenic with a transgenic infested plant subplot (infested minus infested, P1 minus P1, etc.). The same was done for the number of larvae recovered from the straight nontransgenic and nontransgenic with an infested transgenic rootworm-resistant plant. The subtracted data were analyzed as a randomized complete block split-split-plot in space as outlined in Steel et al.

Table 4. Number of western corn rootworm larvae recovered in 2002 from varying configurations of MON863 and isoline plants when 1,500 viable eggs were placed on the infested (Inf) plant

Date	Plant	Transgenic	Trans w/non Inf	Nontrans	Nontrans w/trans Inf	Trans sub	Nontrans sub
12 June	Inf	0.6 ± 0.2cAm	23.2 ± 6.4bAm	32.0 ± 14.6aAm	3.0 ± 1.1cAm	22.6 ± 6.3aAm*	−29.0 ± 14.8bCDm*
17 June	Inf	1.0 ± 0.4cAm	16.4 ± 6.6bBm	33.8 ± 15.9aAm	1.0 ± 0.4cAn	15.4 ± 6.4aABm*	−32.8 ± 15.9bDo*
20 June	Inf	0.4 ± 0.2cAm	14.2 ± 1.7bBm	24.2 ± 6.3aBm	1.4 ± 0.9cAm	13.8 ± 1.7aAm*	−22.8 ± 5.4bDn*
24 June	Inf	0.4 ± 0.2bAm	5.0 ± 2.1abCm	7.4 ± 1.2aCm	1.4 ± 0.5abAm	4.6 ± 2.0aBm*	−6.0 ± 0.9bBCn*
28 June	Inf	0.4 ± 0.2aAm	3.4 ± 1.2aCm	1.8 ± 0.6aCDm	0.6 ± 0.4aAm	3.0 ± 1.3aBm*	−1.2 ± 0.7bABm
3 July	Inf	0.2 ± 0.2aAm	0.2 ± 0.2aCm	0.4 ± 0.2aDm	0.2 ± 0.2aAm	0.0 ± 0.0aCm	−0.2 ± 0.2aAm
12 June	P1	0.2 ± 0.2aAm	0.4 ± 0.2aAn	3.2 ± 1.6aABn	0.8 ± 0.2aBm	0.2 ± 0.2aABn	−2.4 ± 1.5aBm
17 June	P1	0.6 ± 0.2bAm	1.0 ± 0.6bAn	2.4 ± 1.3bABn	10.4 ± 4.7aAm	0.4 ± 0.7aABn	8.0 ± 5.4aAm*
20 June	P1	0.0 ± 0.0bAm	3.8 ± 2.0abAn	6.4 ± 2.4aAn	4.4 ± 2.7abABm	3.8 ± 2.0aAn*	−2.0 ± 3.6bBm
24 June	P1	0.4 ± 0.2aAm	1.2 ± 0.4aAm	5.4 ± 2.2aABmn	3.8 ± 1.5aBm	0.8 ± 0.6aABmn	−1.6 ± 3.4aBm
28 June	P1	1.8 ± 0.6aAm	1.8 ± 1.3aAm	1.0 ± 0.3aABm	1.0 ± 0.5aBm	0.0 ± 1.5aBn	0.0 ± 0.5aBm
3 July	P1	0.4 ± 0.2aAm	0.4 ± 0.2aAm	0.2 ± 0.2aBm	1.2 ± 1.0aBm	0.0 ± 0.3aBm	1.0 ± 1.0aABm
12 July	P2	0.2 ± 0.2aAm	0.0 ± 0.0aAn	0.8 ± 0.2aAn	1.8 ± 1.4aAm	−0.2 ± 0.2aABn	1.0 ± 1.5aAm
17 June	P2	0.4 ± 0.4aAm	1.8 ± 1.8aAn	1.6 ± 0.8aAn	0.8 ± 0.4aAn	1.4 ± 1.9aABn	−0.8 ± 0.7aAn
20 June	P2	0.2 ± 0.2aAm	1.6 ± 0.4aAn	1.6 ± 1.4aAn	0.4 ± 0.2aAm	1.4 ± 0.4aAn*	−1.2 ± 1.5aAm
24 June	P2	0.2 ± 0.2aAm	0.6 ± 0.2aAm	2.2 ± 0.9aAmn	2.6 ± 1.4aAm	0.4 ± 0.2aABn	0.4 ± 1.7aAm
28 June	P2	1.2 ± 1.0aAm	0.0 ± 0.0aAm	0.2 ± 0.2aAm	1.4 ± 0.4aAm	−1.2 ± 1.0bBn	1.2 ± 0.4aAm
3 July	P2	0.2 ± 0.2aAm	0.6 ± 0.6aAm	0.6 ± 0.4aAm	0.2 ± 0.2aAm	0.4 ± 0.4aABm	−0.4 ± 0.5aAm
12 June	P3	0.4 ± 0.2aAm	0.0 ± 0.0aAn	2.0 ± 1.8aAn	1.2 ± 0.7aAm	−0.4 ± 0.2aAn	−0.8 ± 2.2aAm
17 June	P3	0.4 ± 0.2aAm	0.2 ± 0.2aAn	0.2 ± 0.2aAn	1.6 ± 0.9aAn	−0.2 ± 0.4aAn	1.4 ± 0.9aAmn
20 June	P3	0.4 ± 0.4aAm	1.6 ± 0.9aAn	2.0 ± 2.0aAn	1.0 ± 0.4aAm	1.2 ± 1.2aAn*	−1.0 ± 2.3aAm
24 June	P3	0.2 ± 0.2aAm	0.2 ± 0.2aAm	1.8 ± 1.1aAmn	1.4 ± 1.2aAm	0.0 ± 0.3aAn	−0.4 ± 1.4aAm
28 June	P3	0.2 ± 0.2aAm	0.8 ± 0.8aAm	1.4 ± 1.2aAm	0.8 ± 0.5aAm	0.6 ± 0.9aAmn	−0.6 ± 1.0aAm
3 July	P3	0.2 ± 0.2aAm	0.4 ± 0.4aAm	0.6 ± 0.4aAm	0.6 ± 0.6aAm	0.2 ± 0.5aAm	0.0 ± 0.8aAm
12 June	Row	0.4 ± 0.2aAm	5.0 ± 5.0aAn	3.2 ± 1.4aAn	2.2 ± 1.7aAm	4.6 ± 5.1aAn	−1.0 ± 1.1aAm
17 June	Row	1.0 ± 0.8aAm	0.2 ± 0.2aAn	0.2 ± 0.2aAn	1.4 ± 1.2aAn	−0.8 ± 0.8aAn	1.2 ± 1.2aAmn
20 June	Row	1.2 ± 1.0aAm	1.6 ± 0.8aAn	0.8 ± 0.8aAn	0.0 ± 0.0aAm	0.4 ± 1.3aAn	−0.8 ± 0.8aAm
24 June	Row	0.2 ± 0.2aAm	0.4 ± 0.2aAm	1.0 ± 0.6aAn	0.4 ± 0.2aAm	0.2 ± 0.2aAn	−0.6 ± 0.8aAm
28 June	Row	0.8 ± 0.4aAm	0.0 ± 0.0aAm	0.8 ± 0.4aAm	1.0 ± 0.6aAm	−0.8 ± 0.4aAn	0.2 ± 0.6aAm
3 July	Row	0.0 ± 0.0aAm	0.4 ± 0.2aAm	0.0 ± 0.0aAm	0.2 ± 0.2aAm	0.4 ± 0.2aAm	0.2 ± 0.2aAm
12 June	Cnt	0.0 ± 0.0aAm	0.8 ± 0.6aAn	0.8 ± 0.4aAn	0.8 ± 0.5aAm	0.8 ± 0.6aAn	0.0 ± 0.5aABm
17 June	Cnt	0.6 ± 0.4aAm	0.2 ± 0.2aAn	0.0 ± 0.0aAn	3.4 ± 2.3aAn	−0.4 ± 0.5bAn	3.4 ± 2.3aAm*
20 June	Cnt	0.4 ± 0.2aAm	1.0 ± 0.8aAn	0.4 ± 0.4aAn	0.8 ± 0.6aAm	0.6 ± 0.9aAn	0.4 ± 0.8aABm
24 June	Cnt	0.8 ± 0.6aAm	0.4 ± 0.2aAm	1.0 ± 0.6aAn	0.4 ± 0.2aAm	−0.4 ± 0.7aAn	−0.6 ± 0.4aBm
28 June	Cnt	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.4 ± 0.2aAm	0.8 ± 0.4aAm	0.0 ± 0.0aAn	0.4 ± 0.6aABm
3 July	Cnt	0.0 ± 0.0aAm	0.2 ± 0.2aAm	0.0 ± 0.0aAm	0.0 ± 0.0aAm	0.2 ± 0.2aAm	0.0 ± 0.0aABm

Although untransformed data are shown, statistics were performed using log $(x + 1)$ data. Different uppercase letters indicate a significant difference within a column and plant. Different lowercase letters starting with "m" indicate a significant difference between plants, but within a column and date. Different lowercase letters starting with an "a" indicate a significant difference within a row (between treatments, but within a date and plant), either 1) among the third to sixth columns or 2) between the seventh and eighth column. However, because of the experimental design, specific comparisons of the third to fourth columns or the fifth to sixth columns are not appropriate. Significant differences for these comparisons are indicated by an * in the seventh or eighth columns, respectively (863 sub = column 4 minus column 3; Iso sub = column 6 minus column 5).

(1997). Because the treatments were arranged as a 6 by 2 by 6 (sample date × pedigree combination × plant category) factorial, the linear statistical model contained the main plot effect of sample date, the subplot effect of pedigree combination, the sub-subplot of plant category, and all possible interactions of sample date × pedigree combination × plant category. Replications within dates served as the denominator of *F* for testing the effects of dates. Replications within pedigree combinations and sample dates were used as the denominator of *F* for testing pedigree combination and the interaction of pedigree combination × sample date. All other effects used the residual error for the denominator. Beyond the standard ANOVA, we preplanned to compare pedigree combinations within plant category and sampling dates, plant categories within pedigree combination and sampling dates, and sampling dates within pedigree combinations and plant category. This was done with the *t*-test output from PROC MIXED. Analysis of subtracted data was repeated with plant damage and total weight of larvae recovered. Although untransformed data are shown in the tables, all data were transformed by log $(x + 1)$ for analyses to meet the assumptions of equal variance. Too many missing values were present for analysis of subtracted average larval weight data, so some comparisons are not possible.

Results and Discussion

In 2001, main effects for dates, plant category, their interaction, and the interaction of plant category and pedigree combination significantly affected the number of larvae recovered (Table 1). The main effect of pedigree combination did not significantly affect the number of larvae recovered in 2001. In 2002, main effects for dates, plant category, pedigree combinations, and all possible interactions significantly affected the number of larvae recovered (Table 2). The effect of replication was not significant for either year in any analysis. In 2002, 240 plants were checked with gene checks. Of these, 180 should have been positive

Table 5. Plant damage in 2001 from varying configurations of MON863 and isoline plants when 1,500 viable eggs were placed on the infested (Inf) plant

Date	Plant	Transgenic	Trans w/non Inf	Nontrans	Nontrans w/trans Inf	Trans sub	Nontrans sub
18 June	Inf	0.04 ± 0.02aBCm	0.00 ± 0.00aCm	0.01 ± 0.01aDm	0.00 ± 0.00aBm	−0.04 ± 0.02aCDm	−0.01 ± 0.01aAm
22 June	Inf	0.00 ± 0.00aCm	0.03 ± 0.03aCm	0.02 ± 0.01aCDm	0.01 ± 0.01aBm	0.02 ± 0.03aCm	−0.01 ± 0.01aAm
26 June	Inf	0.00 ± 0.00bCm	0.13 ± 0.07abCm	0.06 ± 0.02abCDm	0.25 ± 0.25aAm	0.13 ± 0.07aBCm	0.20 ± 0.24aAm
2 July	Inf	0.44 ± 0.26aAm	0.15 ± 0.12bCm	0.25 ± 0.25abCm	0.26 ± 0.14abAm	−0.29 ± 0.35bDm*	0.00 ± 0.35aAm
6 July	Inf	0.01 ± 0.00cCm	0.41 ± 0.22bBm	0.88 ± 0.26aBm	0.01 ± 0.01cBm	0.40 ± 0.23aBm*	−0.87 ± 0.26bBn*
12 July	Inf	0.26 ± 0.25bABm	1.50 ± 0.00aAm	1.50 ± 0.20aAm	0.40 ± 0.37bAm	1.24 ± 0.25aAm*	−1.10 ± 0.23bBo*
18 June	P1	0.01 ± 0.01aBm	0.02 ± 0.01aBm	0.00 ± 0.00aCm	0.00 ± 0.00aAm	0.01 ± 0.02aAm	0.00 ± 0.00aAm
22 June	P1	0.01 ± 0.01aBm	0.01 ± 0.01aBm	0.03 ± 0.01aCm	0.01 ± 0.01aAm	0.00 ± 0.01aAm	−0.02 ± 0.02aAm
26 June	P1	0.01 ± 0.01aBm	0.01 ± 0.01aBm	0.01 ± 0.01aCm	0.03 ± 0.03aAmn	0.01 ± 0.01aAm	0.02 ± 0.03aAm
2 July	P1	0.03 ± 0.01aBn	0.01 ± 0.00aBm	0.01 ± 0.00aCn	0.02 ± 0.01aAn	−0.02 ± 0.01aAm	0.01 ± 0.01aAm
6 July	P1	0.03 ± 0.03bBm	0.01 ± 0.01bBn	0.28 ± 0.13aBn	0.14 ± 0.12abAm	−0.02 ± 0.03aAn	−0.14 ± 0.23aABm
12 July	P1	0.53 ± 0.49abAm	0.26 ± 0.25bcAn	0.57 ± 0.21aAn	0.15 ± 0.12cAmn	−0.28 ± 0.24aAo	−0.42 ± 0.14aBn*
18 June	P2	0.00 ± 0.00aAm	0.01 ± 0.01aAm	0.00 ± 0.00aAm	0.00 ± 0.00aAm	0.01 ± 0.01aAm	0.00 ± 0.00aAm
22 June	P2	0.00 ± 0.00aAm	0.00 ± 0.00aAm	0.00 ± 0.00aAm	0.01 ± 0.01aAm	0.00 ± 0.00aAm	0.01 ± 0.01aAm
26 June	P2	0.00 ± 0.00aAm	0.01 ± 0.01aAm	0.00 ± 0.00aAm	0.00 ± 0.00aAn	0.01 ± 0.01aAm	0.00 ± 0.00aAm
2 July	P2	0.03 ± 0.02aAn	0.01 ± 0.01aAm	0.01 ± 0.01aAn	0.01 ± 0.01aAn	−0.03 ± 0.02aAm	0.00 ± 0.01aAm
6 July	P2	0.00 ± 0.00aAm	0.03 ± 0.03aAn	0.00 ± 0.00aAo	0.03 ± 0.02aAm	0.03 ± 0.03aAn	0.03 ± 0.02aAm
12 July	P2	0.03 ± 0.02aAn	0.13 ± 0.13aAno	0.08 ± 0.06aAo	0.09 ± 0.06aAn	0.10 ± 0.10aAn	0.01 ± 0.01aAm
18 June	P3	0.01 ± 0.01aAm	0.00 ± 0.00aAm	0.00 ± 0.00aAm	0.00 ± 0.00aAm	0.00 ± 0.01aAm	0.00 ± 0.00aAm
22 June	P3	0.07 ± 0.06aAm	0.01 ± 0.00aAm	0.06 ± 0.06aAm	0.00 ± 0.00aAm	−0.06 ± 0.06aAm	−0.06 ± 0.06aAm
26 June	P3	0.02 ± 0.01aAm	0.01 ± 0.01aAm	0.00 ± 0.00aAm	0.02 ± 0.01aAn	−0.01 ± 0.01aAm	0.02 ± 0.01aAm
2 July	P3	0.06 ± 0.06aAn	0.00 ± 0.00aAm	0.01 ± 0.00aAn	0.01 ± 0.01aAn	−0.06 ± 0.06aAm	0.00 ± 0.01aAm
6 July	P3	0.00 ± 0.00aAm	0.01 ± 0.01aAn	0.00 ± 0.00aAo	0.01 ± 0.01aAm	0.01 ± 0.01aAn	0.01 ± 0.01aAm
12 July	P3	0.02 ± 0.01aAn	0.00 ± 0.00aAo	0.01 ± 0.01aAo	0.06 ± 0.06aAn	−0.02 ± 0.01aAno	0.06 ± 0.06aAm
18 June	Row	0.00 ± 0.00aAm	0.02 ± 0.01aAm	0.00 ± 0.00aAm	0.00 ± 0.00aAm	0.02 ± 0.01aAm	0.00 ± 0.00aAm
22 June	Row	0.00 ± 0.00aAm	0.03 ± 0.03aAm	0.00 ± 0.00aAm	0.00 ± 0.00aAm	0.03 ± 0.03aAm	0.00 ± 0.00aAm
26 June	Row	0.01 ± 0.01aAm	0.01 ± 0.01aAm	0.00 ± 0.00aAm	0.03 ± 0.03aAmn	0.00 ± 0.01aAm	0.02 ± 0.02aAm
2 July	Row	0.00 ± 0.00aAn	0.00 ± 0.00aAm	0.00 ± 0.00aAn	0.00 ± 0.00aAn	0.00 ± 0.00aAm	0.00 ± 0.00aAm
6 July	Row	0.00 ± 0.00aAm	0.01 ± 0.01aAn	0.03 ± 0.02aAo	0.01 ± 0.01aAm	0.01 ± 0.01aAn	−0.02 ± 0.03aAm
12 July	Row	0.01 ± 0.01aAn	0.00 ± 0.00aAo	0.04 ± 0.01aAo	0.01 ± 0.01aAn	−0.01 ± 0.01aAno	−0.02 ± 0.02aAm
18 June	Cnt	0.01 ± 0.00aAm	0.00 ± 0.00aAm	0.00 ± 0.00aAm	0.00 ± 0.00aAm	−0.01 ± 0.00aAm	0.00 ± 0.00aAm
22 June	Cnt	0.07 ± 0.06aAm	0.01 ± 0.01aAm	0.03 ± 0.03aAm	0.00 ± 0.00aAm	−0.06 ± 0.06aAm	−0.02 ± 0.03aAm
26 June	Cnt	0.03 ± 0.03aAm	0.01 ± 0.01aAm	0.00 ± 0.00aAm	0.00 ± 0.00aAn	−0.02 ± 0.02aAm	0.00 ± 0.00aAm
2 July	Cnt	0.01 ± 0.00aAn	0.00 ± 0.00aAm	0.00 ± 0.00aAn	0.02 ± 0.01aAn	−0.01 ± 0.00aAm	0.01 ± 0.01aAm
6 July	Cnt	0.00 ± 0.00aAm	0.01 ± 0.01aAn	0.02 ± 0.01aAo	0.07 ± 0.06aAm	0.01 ± 0.01aAn	0.05 ± 0.06aAm
12 July	Cnt	0.03 ± 0.01aAn	0.01 ± 0.01aAo	0.04 ± 0.02aAo	0.03 ± 0.02aAn	−0.02 ± 0.01aAno	−0.01 ± 0.01aAm

Plant damage was assessed using the node-injury scale (Olson et al. 2005). Although untransformed data are shown, statistics were performed using log $(x + 1)$ data. Different uppercase letters indicate a significant difference within a column and plant. Different lowercase letters starting with "m" indicate a significant difference between plants, but within a column and date. Different lowercase letters starting with an "a" indicate a significant difference within a row (between treatments, but within a date and plant), either 1) among the third to sixth columns or 2) between the seventh and eighth column. However, because of the experimental design, specific comparisons of the third to fourth columns or the fifth to sixth columns are not appropriate. Significant differences for these comparisons are indicated by an * in the seventh or eighth columns, respectively (863 sub = column 4 minus column 3; Iso sub = column 6 minus column 5).

and 60 should have been negative. In each case, the gene check results were as expected.

One of objectives of the current study was to determine the extent of plant-to-plant movement that would occur between nontransgenic and transgenic rootworm-resistant roots and vice versa. Either very little movement from nontransgenic-to-transgenic rootworm-resistant roots occurred or transgenic rootworm-resistant corn was more efficacious against later instars in our study than was expected. The number of larvae recovered from transgenic rootworm-resistant plants adjacent to infested, nontransgenic plants was not statistically significant in either year of the study. Specifically, the number of larvae recovered from P1, transgenic rootworm-resistant plants that were adjacent to an infested, nontransgenic plant did not significantly change over sampling dates in 2001 or 2002 (Tables 3 and 4). In fact, in 2001, the number of larvae that moved from infested, nontransgenic plants to P1 transgenic rootworm-resistant plants was significantly fewer than the number of larvae that moved from infested, nontransgenic plants to P1 nontransgenic plants for the third sample date (Table 3). and this difference was also statistically significant the second sampling date for P2 plants (Table 3). On the second sampling date in 2002, the difference between the number of larvae on P1 nontransgenic plants adjacent to infested transgenic rootworm-resistant plants and P1 nontransgenic plants adjacent to infested nontransgenic plants was significant (Table 4), implying that neonate western corn rootworm larvae may be repelled by transgenic rootworm-resistant plants. This difference was not significant in 2001 (Table 3).

The total number of larvae recovered per plant was higher in 2002 than in 2001, but general trends were similar in both years. Most larvae were recovered on the infested plant, especially when that plant was a nontransgenic plant (Tables 3 and 4). The next highest number was recovered from the P1 plant followed by the P2 plant. Similarly, plant damage was highest on the infested plant followed by damage to the P1 plant (Tables 5 and 6). Plant damage was also almost always

Table 6. Plant damage in 2002 from varying configurations of Yieldgard Rootworm and isoline plants when 1,500 viable eggs were placed on the infested (Inf) plant

Date	Plant	Transgenic	Trans w/non Inf	Nontrans	Nontrans w/trans Inf	Trans sub	Nontrans sub
12 June	Inf	0.00 ± 0.00aAm	0.02 ± 0.01aCm	0.02 ± 0.01aCm	0.00 ± 0.00aAm	0.02 ± 0.01aCm	−0.02 ± 0.01aAm
17 June	Inf	0.00 ± 0.00aAm	0.08 ± 0.05aCm	0.09 ± 0.04aCm	0.00 ± 0.00aAm	0.08 ± 0.04aCm	−0.08 ± 0.04aABm
20 June	Inf	0.01 ± 0.00bAm	0.50 ± 0.21aBm	0.42 ± 0.19aBm	0.00 ± 0.00bAm	0.49 ± 0.21aBm*	−0.42 ± 0.19bBCn*
24 June	Inf	0.01 ± 0.00bAm	0.35 ± 0.17aBm	0.52 ± 0.16aBm	0.01 ± 0.00bAm	0.35 ± 0.17aBm*	−0.51 ± 0.17bCn*
28 June	Inf	0.01 ± 0.00cAm	0.41 ± 0.16bBm	0.95 ± 0.28aAm	0.00 ± 0.00cAn	0.41 ± 0.16aBm*	−0.95 ± 0.28bDp*
3 July	Inf	0.02 ± 0.01cAm	1.35 ± 0.32aAm	1.05 ± 0.15bAm	0.06 ± 0.05cAn	1.33 ± 0.33aAm*	−0.99 ± 0.14bDn*
12 June	P1	0.00 ± 0.00aAm	0.00 ± 0.00aAm	0.01 ± 0.00aBm	0.05 ± 0.05aBm	0.00 ± 0.00aAm	0.04 ± 0.05aAm
17 June	P1	0.00 ± 0.00aAm	0.00 ± 0.00aAm	0.05 ± 0.02aBm	0.01 ± 0.00aBm	0.00 ± 0.00aAm	−0.04 ± 0.02aAm
20 June	P1	0.00 ± 0.00aAm	0.01 ± 0.01aAn	0.09 ± 0.04aBn	0.13 ± 0.09aBm	0.01 ± 0.01aAn	0.04 ± 0.11aAm
24 June	P1	0.00 ± 0.00bAm	0.01 ± 0.00bAn	0.38 ± 0.23aAm	0.04 ± 0.02bBm	0.01 ± 0.01aAn	−0.34 ± 0.22bBn*
28 June	P1	0.00 ± 0.00bAm	0.06 ± 0.05bAn	0.37 ± 0.18aAno	0.18 ± 0.09abABmn	0.06 ± 0.05aAn	−0.19 ± 0.10bABno*
3 July	P1	0.01 ± 0.00bAm	0.04 ± 0.02bAn	0.37 ± 0.11aAn	0.38 ± 0.17aAm	0.03 ± 0.02aAn	0.01 ± 0.20aAm
12 June	P2	0.00 ± 0.00aAm	0.00 ± 0.00aAm	0.00 ± 0.00aBm	0.05 ± 0.04aBm	0.00 ± 0.00aAm	0.05 ± 0.04aAm
17 June	P2	0.00 ± 0.00aAm	0.00 ± 0.00aAm	0.02 ± 0.01aBm	0.00 ± 0.00aBm	0.00 ± 0.00aAm	−0.01 ± 0.01aAm
20 June	P2	0.05 ± 0.02aAm	0.00 ± 0.00aAn	0.01 ± 0.00aBn	0.02 ± 0.01aBm	−0.05 ± 0.02aAn	0.01 ± 0.01aAm
24 June	P2	0.00 ± 0.00aAm	0.01 ± 0.00aAn	0.02 ± 0.01aBn	0.04 ± 0.02aBm	0.01 ± 0.00aAn	0.02 ± 0.02aAm
28 June	P2	0.02 ± 0.01bAm	0.05 ± 0.05bAn	0.29 ± 0.18aAno	0.28 ± 0.18aAm	0.04 ± 0.05aAn	−0.01 ± 0.05aAm
3 July	P2	0.01 ± 0.00aAm	0.05 ± 0.05aAn	0.13 ± 0.05aABo	0.09 ± 0.04aABn	0.05 ± 0.05aAn	−0.04 ± 0.07aAm
12 June	P3	0.00 ± 0.00aAm	0.00 ± 0.00aAm	0.03 ± 0.01aAm	0.00 ± 0.00aAm	0.00 ± 0.00aAm	0.00 ± 0.00aAm
17 June	P3	0.01 ± 0.00aAm	0.00 ± 0.00aAm	0.02 ± 0.01aAm	0.01 ± 0.00aAm	0.00 ± 0.00aAm	−0.01 ± 0.01aAm
20 June	P3	0.01 ± 0.00aAm	0.03 ± 0.02aAn	0.03 ± 0.01aAn	0.01 ± 0.00aAm	0.02 ± 0.02aAn	−0.02 ± 0.01aAm
24 June	P3	0.01 ± 0.00aAm	0.00 ± 0.00aAn	0.04 ± 0.02aAn	0.03 ± 0.02aAm	0.00 ± 0.01aAn	−0.01 ± 0.02aAm
28 June	P3	0.01 ± 0.00aAm	0.02 ± 0.01aAn	0.08 ± 0.04aAP	0.15 ± 0.09aAmn	0.01 ± 0.01aAn	0.07 ± 0.12aAm
3 July	P3	0.01 ± 0.00aAm	0.01 ± 0.00aAn	0.16 ± 0.10aAo	0.05 ± 0.02aAn	0.00 ± 0.00aAn	−0.10 ± 0.11aAm
12 June	Row	0.00 ± 0.00aAm	0.00 ± 0.00aAm	0.02 ± 0.01aBm	0.00 ± 0.00aAm	0.00 ± 0.00aAm	−0.01 ± 0.01aAm
17 June	Row	0.00 ± 0.00aAm	0.00 ± 0.00aAm	0.02 ± 0.01aBm	0.03 ± 0.01aAm	0.00 ± 0.00aAm	0.01 ± 0.01aAm
20 June	Row	0.00 ± 0.00aAm	0.01 ± 0.01aAn	0.02 ± 0.00aBn	0.07 ± 0.05aAm	0.01 ± 0.01aAn	0.06 ± 0.05aAm
24 June	Row	0.00 ± 0.00aAm	0.02 ± 0.02aAn	0.11 ± 0.10aBn	0.04 ± 0.02aAm	0.02 ± 0.02aAn	−0.07 ± 0.10aAm
28 June	Row	0.00 ± 0.00bAm	0.07 ± 0.05bAn	0.55 ± 0.29aAn	0.08 ± 0.04bAmn	0.06 ± 0.04aAn	−0.47 ± 0.32bBo*
3 July	Row	0.03 ± 0.02aAm	0.00 ± 0.00aAn	0.04 ± 0.01aBo	0.03 ± 0.01aAn	−0.03 ± 0.02aAn	−0.01 ± 0.01aAm
12 June	Cnt	0.00 ± 0.00aAm	0.00 ± 0.00aBm	0.02 ± 0.01aBm	0.01 ± 0.00aAm	0.00 ± 0.00aAm	−0.01 ± 0.01aAm
17 June	Cnt	0.00 ± 0.00aAm	0.00 ± 0.00aBm	0.02 ± 0.01aBm	0.02 ± 0.01aAm	0.00 ± 0.00aAm	0.00 ± 0.01aAm
20 June	Cnt	0.00 ± 0.00aAm	0.00 ± 0.00aBn	0.02 ± 0.01aBn	0.02 ± 0.00aAm	0.00 ± 0.00aAn	0.00 ± 0.01aAm
24 June	Cnt	0.01 ± 0.01aAm	0.02 ± 0.02aABn	0.02 ± 0.01aBn	0.04 ± 0.02aAm	0.01 ± 0.02aAn	0.03 ± 0.02aAm
28 June	Cnt	0.02 ± 0.01bAm	0.21 ± 0.20abAn	0.23 ± 0.11aAop	0.05 ± 0.02abAmn	0.19 ± 0.19aAn*	−0.18 ± 0.09bAno*
3 July	Cnt	0.00 ± 0.00aAm	0.00 ± 0.00aBn	0.04 ± 0.02aABo	0.07 ± 0.04aAn	0.00 ± 0.00aAn	0.03 ± 0.05aAm

Plant damage was assessed by using the node-injury scale (Olson et al. 2005). Although untransformed data are shown, statistics were performed using log $(x + 1)$ data. Different uppercase letters indicate a significant difference within a column and plant. Different lowercase letters starting with "m" indicate a significant difference between plants, but within a column and date. Different lowercase letters starting with an "a" indicate a significant difference within a row (between treatments, but within a date and plant), either 1) among the third to sixth columns or 2) between the seventh and eighth column. However, because of the experimental design, specific comparisons of the third to fourth columns or the fifth to sixth columns are not appropriate. Significant differences for these comparisons are indicated by an * in the seventh or eighth columns, respectively (863 sub = column 4 minus column 3; Iso sub = column 6 minus column 5).

highest on the last sampling date. This was the case despite the fact that very few larvae were recovered on the last several infestation dates (Tables 3 and 4). Hibbard et al. (2003, 2004) also recovered very few western corn rootworm larvae on the last sample dates, yet the roots sampled on these dates were the most damaged. Apparently, most of the damage to corn roots that is detected by damage ratings takes place shortly before pupation.

Average wet weight of western corn rootworm larvae also was evaluated in both years. In 2001, only the main effect for dates significantly affected average larval weight (Table 1). In 2002, dates, plants, and the interaction of dates by pedigree combinations significantly affected average larval weight (Table 2). The main effect of pedigree combinations did not significantly affect average weight in either 2001 or 2002 (Tables 1 and 2). When comparing specific plant categories and dates for average weight, the average weights of western corn rootworm larvae recovered from nontransgenic and transgenic rootworm-resistant roots were not significantly different, except on the first sample date with P2 plants in 2002, when the average weight of those recovered from transgenic rootworm-resistant plants was significantly higher than those recovered from nontransgenic roots (Table 7). Apparently, if western corn rootworm larvae are able to establish on transgenic rootworm-resistant plants, larval growth is relatively normal.

In 2001, only a total of four morphologically identified southern corn rootworm larvae were found among nearly 500 larvae recovered. In 2002, the total number of larvae morphologically distinguished as western corn rootworm larvae and southern corn rootworm larvae from the straight nontransgenic pedigree alone was 709 and 342, respectively. The number of larvae morphologically distinguished as western corn rootworm larvae and southern corn rootworm larvae from the straight transgenic rootworm-resistant pedigree was 80 and 35, respectively. An average of 8.86 western corn rootworm larvae were recovered from nontransgenic plants for every western corn

Table 7. Average wet weight (milligrams) of western corn rootworm larvae recovered in 2002 from varying configurations of MON863 and isoline plants when 1,500 viable eggs were placed on the infested (Inf) plant

Date	Plant	Transgenic	Trans w/non Inf	Nontransgenic	Nontransgenic w/trans Inf
12 June	Inf	0.21 ± 0.12aBn	0.32 ± 0.08aBm	0.34 ± 0.14aDo	1.07 ± 0.97aBCm
17 June	Inf	2.40 ± 1.11aBmn	0.56 ± 0.14abABm	0.75 ± 0.11abCDm	0.08 ± 0.03bCm
20 June	Inf	1.90 ± 0.30aBm	2.22 ± 0.55aAm	2.77 ± 0.56aBCm	7.53 ± 6.89aABm
24 June	Inf	0.45 ± 0.08aBm	1.47 ± 0.40aABn	2.75 ± 0.48aBCm	0.83 ± 0.23aBCn
28 June	Inf	0.81 ± 0.19bBm	3.01 ± 0.95abAm	3.73 ± 1.06abABmn	10.02 ± 7.89aAm
3 July	Inf	12.20 ± *aAmn	0.66 ± *bABn	12.47 ± 6.86aAm	2.71 ± *abABCm
12 June	P1	0.27 ± *aABmn	0.27 ± 0.07aBm	0.89 ± 0.47aCo	1.83 ± 1.71aBm
17 June	P1	0.21 ± 0.17aBo	0.63 ± 0.13aBm	1.09 ± 0.34aBCm	1.20 ± 0.14aBm
20 June	P1	*	2.71 ± 1.13aABm	3.11 ± 0.48aABm	12.67 ± 11.52aAm
24 June	P1	0.49 ± aABm	1.97 ± 0.96aABmn	2.60 ± 0.70aABCm	2.36 ± 0.47aABmn
28 June	P1	1.76 ± 0.56aABm	2.69 ± 0.68aABm	4.16 ± 1.07aABmn	5.45 ± 1.32aABm
3 July	P1	7.29 ± 6.40aAn	6.37 ± 0.09aAmn	8.81 ± *aAm	1.84 ± 0.64aBm
12 June	P2	5.64 ± *aAm	*	0.49 ± 0.32bBo	0.45 ± 0.19abBm
17 June	P2	1.04 ± *aAmno	0.51 ± *aAm	4.00 ± 2.93aABm	1.42 ± 0.19aABm
20 June	P2	0.75 ± *aAm	1.34 ± 0.45aAm	0.84 ± 0.43aABm	3.65 ± 3.26aABm
24 June	P2	0.19 ± *aAm	1.92 ± 1.47aAmn	2.39 ± 0.53aABm	5.01 ± 2.27aAmn
28 June	P2	1.22 ± 0.40aAm	*	4.10 ± *aAmn	5.97 ± 1.91aAm
3 July	P2	3.11 ± *aAmn	2.44 ± *aAmn	3.24 ± 1.77aABm	0.47 ± *aBm
12 June	P3	0.74 ± 0.13aCmn	*	4.98 ± 4.30aABmn	0.36 ± 0.08aBm
17 June	P3	5.94 ± 2.83aABm	0.67 ± *abAm	0.46 ± *abBm	0.99 ± 0.54bBm
20 June	P3	1.24 ± *aBCm	2.78 ± 2.41aAm	0.67 ± *aBm	4.71 ± 1.69aAm
24 June	P3	0.68 ± *bCm	8.27 ± *aAm	3.65 ± 2.03abABm	1.73 ± 1.15abABmn
28 June	P3	1.38 ± *abBCm	3.16 ± *abAm	9.57 ± 5.25aAm	0.90 ± 0.67bBn
3 July	P3	27.89 ± *aAm	1.09 ± *bAn	6.62 ± 3.66abABm	6.20 ± *abAm
12 June	Row	1.23 ± 0.21aABmn	*	2.25 ± 1.96aAno	6.68 ± 6.40aAm
17 June	Row	0.42 ± 0.31aBno	1.67 ± *aABm	0.31 ± *aAm	1.27 ± 0.26aAm
20 June	Row	7.23 ± 6.09aAm	1.56 ± 0.84aBm	2.95 ± *aAm	*
24 June	Row	3.49 ± *aABm	1.77 ± 0.46aABmn	3.62 ± 1.33aAm	5.30 ± 2.09aAm
28 June	Row	6.71 ± 5.11aAm	*	3.34 ± 1.71aAn	6.23 ± 2.02aAm
3 July	Row	*	8.02 ± 1.05aAm	*	4.16 ± *aAm
12 June	Cnt	*	2.26 ± 0.48aABm	8.36 ± 4.45aABm	1.28 ± 0.18aAm
17 June	Cnt	5.06 ± 2.83aAm	5.35 ± *aAm	*	1.57 ± 0.45aAm
20 June	Cnt	1.53 ± 0.79aAm	2.39 ± 2.22aABm	0.51 ± *aBm	8.55 ± 8.13aAm
24 June	Cnt	1.20 ± 0.99aAm	2.39 ± 0.27aABmn	0.88 ± 0.48aBm	0.73 ± 0.02aAn
28 June	Cnt	*	*	11.45 ± *aAm	4.45 ± 2.32aAmn
3 July	Cnt	*	1.72 ± *aBm	*	*

Although untransformed data are shown, statistics were performed using log $(x + 1)$ data. Different uppercase letters indicate a significant difference within a column and plant. Different lowercase letters starting with "m" indicate a significant difference between plants, but within a column and date. Different lowercase letters starting with an "a" indicate a significant difference within a row (between treatments, but within a date and plant). An * indicates that no larvae were recovered. An * after the ± indicates that larvae were recovered from only one of the five replicates. However, because of the experimental design, specific comparisons of the third to fourth columns or the fifth to sixth columns are not appropriate.

rootworm larvae recovered from transgenic rootworm-resistant plants. An average of 9.77 southern corn rootworm larvae were recovered from nontransgenic plants for every southern corn rootworm recovered from transgenic rootworm-resistant plants. Although Monsanto Company does not make specific claims that transgenic rootworm-resistant plants are efficacious against southern corn rootworm larvae (EPA Scientific Advisory Panel 2002), in our trial, transgenic rootworm-resistant plants were as effective against southern corn rootworm larvae as against western corn rootworm larvae in 2002.

In total, DNA was successfully extracted from 38 of 60 and 45 of 60 larvae morphologically identified as western corn rootworm from nontransgenic and transgenic rootworm-resistant plants, respectively. The reason that DNA was not obtained from more of the samples was most likely because of degradation of the samples before storage in ethanol (some larvae were in water for up to 24 h in warm, summer greenhouse conditions). PCR amplification of the COI gene was conducted for all successful DNA extractions, including the previously identified controls. The resulting COI amplicon was 625 bp (Fig. 3). Digestion of the amplicon with the *Alu*I restriction enzyme resulted in individual fragment patterns that were diagnostic for each respective species (Fig. 3). Known western corn rootworm had identifiable fragment sizes of 426, 357, 277, 188, and 138 bp, whereas southern corn rootworm had fragment sizes of 416 and 147 bp. As a control, we included a known northern corn rootworm that had a single fragment of 579 bp. All species had some fragments below 100 bp; however, fragments below this size were difficult to resolve and were eliminated from consideration. The fragment sizes for both western and southern corn rootworm added up to more than our starting template of 625 bp, due to incomplete digestion of the amplicon by *Alu*I. The process was repeated five times with both known and unknown samples yielding the same results. Of the 83 (38 from nontransgenic, 45 from transgenic rootworm-resistant plants) morphologically identified western corn rootworm larvae where DNA extraction and subsequent PCR-restriction fragment-length polymorphism was

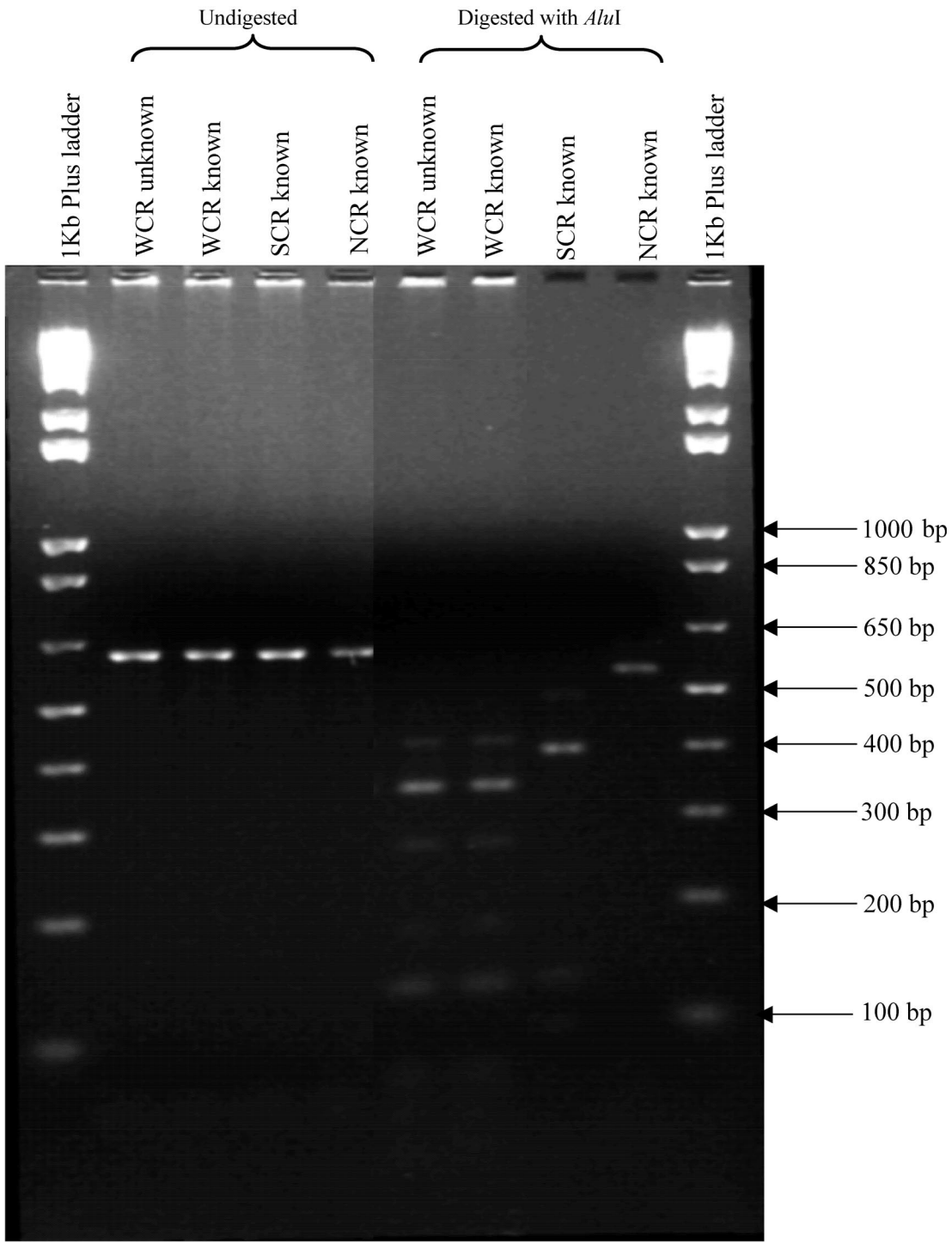

Fig. 3. Agarose gel (2%) of undigested and digested mitochondrial cytochrome oxidase subunit I amplicon for species determination of recovered larvae stained with ethidium bromide visualized over a UV transilluminator.

successful, 69 (32 from nontransgenic, 37 from transgenic rootworm-resistant plants) or 82.1% were actually western corn rootworm. No species beyond either western corn rootworm or southern corn rootworm was detected in the assay. It was previously known that there is no method to discriminate morphologically between neonate western and southern corn rootworm larvae (Krysan 1986). In our study, many of the

larvae morphologically identified as western corn rootworm that were actually southern corn rootworm larvae were first instars, but this was not the case for all. The morphological characters for separating larvae of these two species as outlined by Mendoza and Peters (1964) and Krysan (1986) are far from perfect.

Davis and Onstad (2000) evaluated movement of European corn borer, *Ostrinia nubilalis* (Hübner), larvae in different combinations of Bt corn with resistance to feeding from the European corn borer and nontransgenic corn. They found increased neonate dispersal away from Bt plants and low incidence of late instar movement from non-Bt plants to Bt plants. They also found reduced survival of those larvae that moved from Bt plants to non-Bt plants. Computer simulations based on these and other data led them to conclude that resistant European corn borer populations will likely develop faster in seed mixtures compared with separate plantings of Bt and non-Bt corn. It should be noted that the dose of Cry1Ab endotoxin present in the MON 810 plants used by Davis and Onstad (2000) has been classified as a high dose. The February 1998 Scientific Advisory Panel defined a high dose for lepidopteran-active Bt proteins as 25 times the amount of Bt δ-endotoxin necessary to kill susceptible individuals (EPA Scientific Advisory Panel 1998).

In data submitted to the EPA as part of its registration packet for Cry3Bb1-expressing plants, Monsanto Company indicated the number of adults produced from plants expressing the Cry3Bb1 protein compared with the number of adults from untreated isoline plants could be anywhere between 17 and 100%. Monsanto also indicated that transgenic rootworm-resistant plants do not control second or third instars. These two facts led the Scientific Advisory Panel evaluating Monsanto's Resistance Management Plant to conclude that Cry3Bb1 was not a high dose product (EPA Scientific Advisory Panel 2002). In their report, the panel also noted that in current efforts to understand adaptation of the western corn rootworm to transgenic corn through simulation modeling, all four models assumed that the majority of beetles currently produced on transgenic rootworm-resistant plants are of a susceptible genotype. Their assumption has not been documented, but if susceptible adults are currently being produced, migration of larvae from alternate hosts or nonexpressing corn plants to complete their development on plants expressing the Cry3Bb1 protein may actually prolong product duration by producing a greater number of susceptible insects from within the transgenic field. If indeed such a resistance management benefit of larval movement were to exist with transgenic rootworm-resistant plants, our data indicate that the possible beneficial effects of this movement would be less than if larvae readily moved to transgenic rootworm-resistant plants from nontransgenic plants. Our data imply that such movement will only occur when nontransgenic plants are highly damaged and although not documented in this manuscript, this also would occur when alternate hosts are killed by postemergence herbicide sprays such as nicosulfuron, primisulfuron, atrazine/oil, or glyphosate.

In summary, the number of western corn rootworm larvae moving to transgenic rootworm-resistant plants from adjacent, infested nontransgenic plants in the current study was low and not statistically significant in either 2001 or 2002. In 2001, significantly fewer larvae were recovered from transgenic rootworm-resistant plants than nontransgenic plants that were both adjacent to infested, nontransgenic plants. In 2002, neonate larvae moved the other way. Significantly more neonate western corn rootworm larvae were recovered from nontransgenic plants adjacent to infested, transgenic rootworm-resistant plants than nontransgenic plants adjacent to infested, nontransgenic plants on the second sample date. Although an alternate hypothesis for the 2001 data could be that these plants may have been more efficacious against later instars in our study than was expected, the sum of all data implies that western corn rootworm larvae prefer nontransgenic plants over transgenic rootworm-resistant plants when both are available. However, when damage to the infested, nontransgenic plant was extremely high, enough western corn rootworm apparently did move to transgenic rootworm-resistant plants to cause significantly more damage on the last sample date in 2001 compared with earlier sample dates and is likely due to these older larvae moving more readily and being less sensitive to the Cry3Bb protein. The full implications of these data toward resistance management plans are yet to be determined and will be influenced by data that are not currently available, such as the selection intensity of transgenic rootworm-resistant plants on neonate and later instars under differing soil and growing conditions, and the scale of refuges put into practice.

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References Cited

Baum, J. A., C. Chu, M. Rupar, G. R. Brown, W. P. Donovan, J. E. Huesing, O. Ilagan, T. M. Malvar, M. Pleau, M. Walters, et al. 2004. Binary toxins from *Bacillus thuringiensis* active against the western corn rootworm, *Diabrotica virgifera virgifera* LeConte. Appl. Environ. Microbiol. 70: 4889–4898.

Clark, T. L., L. J. Meinke, and J. E. Foster. 2001a. Molecular phylogeny of *Diabrotica* beetles (Coleoptera: Chrysomelidae) inferred from analysis of combined mitochondrial and nuclear DNA sequences. Insect Mol. Biol. 10: 303–314.

Clark, T. L., J. E. Foster, and L. J. Meinke. 2001b. PCR-RFLP of mitochondrial COI DNA provides diagnostic markers for selected *Diabrotica* species (Coleoptera: Chrysomelidae) Bull. Entomol. Res. 91: 419–427.

Davis, P. M., and D. W. Onstad. 2000. Seed mixtures as a resistance management strategy for European corn borers (Lepidoptera: Crambidae) infesting transgenic corn expressing Cry1ab protein. J. Econ. Entomol. 93: 937–948.

Ellis, R. T., B. A. Stockhoff, L. Stamp, H. E. Schnepf, G. E. Schwab, M. Knuth, J. Russell, G. A. Cardineau, and K. E. Narva. 2002. Novel proteins active on western corn rootworm, *Diabrotica virgifera virgifera* LeConte. Appl. Environ. Microbiol. 68: 1137–1145.

English, L. H., S. M. Brussock, T. M. Malvar, J. W. Bryson, C. A. Kulesza, F. S. Walters, S. L. Slatin, M. A. Von Tersch, and C. Romano. 2000. Insect-resistant transgenic plants. U.S. patent 6.023:013.

EPA Scientific Advisory Panel Meeting. 1998. Subpanel on *Bacillus thuringiensis* (Bt) plant-pesticides and resistance management meeting held on February 9 and 10. http://www.epa.gov/scipoly/sap/1998/february/finalfeb.pdf.

EPA Scientific Advisory Panel Meeting. 2002. Corn rootworm plant-incorporated protectant non-target insect and insect resistance management issues, part B: insect resistance management issues. http://www.epa.gov/scipoly/sap/2002/august/august2002final.pdf.

Hibbard, B. E., D. P. Duran, M. R. Ellersieck, and M. M. Ellsbury. 2003. Post-establishment movement of western corn rootworm larvae (Coleoptera: Chrysomelidae) in central Missouri corn. J. Econ. Entomol. 96: 599–608.

Hibbard, B. E., M. L. Higdon, D. P. Duran, Y. M. Schweikert, and M. R. Ellersieck. 2004. Role of egg density on establishment and plant-to-plant movement by western corn rootworm larvae (Coleoptera: Chrysomelidae). J. Econ. Entomol. 97: 871–882.

Krysan, J. L. 1986. Introduction: biology, distribution, and identification of pest *Diabrotica*, pp. 1–23. *In* J. L. Krysan and A. T. Miller [eds.], Methods for the study of pest *Diabrotica.* Springer, New York.

Mallet, J., and P. Porter. 1992. Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy? Proc. R. Soc. Lond. B. 250: 165–169.

Mendoza, C. E., and D. C. Peters. 1964. Species differentiation among mature larvae of *Diabrotica undecimpunctata howardi, D. virgifera,* and *D. longicornis.* J. Kans. Entomol. Soc. 37: 123–125.

Moellenbeck, D. J., M. L. Peters, J. W. Bing, J. R. Rouse, L. S. Higgins, L. Sims, T. Nevshemal, L. Marshall, R. T. Ellis, P. G. Bystrak, et al. 2001. Insecticidal proteins from *Bacillus thuringiensis* protect corn from corn rootworms. Nat. Biotech. 19: 668–672.

Oleson, J. D., Y.-L. Park, T. M. Nowatzki, and J. J. Tollefson. 2005. Node-injury scale to evaluate root injury by corn rootworms (Coleoptera: Chrysomelidae). J. Econ. Entomol. 98: 1–8.

Ritchie, W. W., J. J. Hanway, and G. O. Benson. 1992. How a corn plant develops. Iowa State University of Science and Technology Cooperative Extension Service, Special Report 48.

SAS Institute. 1990. SAS/STAT user's guide, version 6, 4th ed., vol. 2. SAS Institute, Cary, NC.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87: 108–110.

Steel, R. G., J. H. Torrie, and D. A. Dickey. 1997. Principles and procedures of statistics: a biometrical approach, 3rd ed. McGraw Hill, New York.

Vaughn, T. T., T. Cavato, G. Brar, T. Coombe, T. DeGooyer, S. Ford, M. Groth, A. Howe, S. Johnson, K. Kolacz, et al. 2005. A method of controlling corn rootworm feeding using a *Bacillus thuringiensis* protein expressed in transgenic maize. Crop Sci. 45: 931–938.

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